

# Conductive Polypyrrole-polycaprolactone Scaffolds for Electrophysiological Maturation of Cardiomyocytes

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**Statement of Purpose:** Current strategies for creating engineered heart tissue are often limited by the formation of cardiomyocytes with immature electrophysiological properties, which may not be suitable for clinical or drug discovery applications. Improving cell-cell signaling via incorporation of conductive materials into biomimetic scaffolds may constitute an important step in the creation of engineered myocardium which recapitulates the native electrical propagation within the heart. However, many conductive materials pose limitations in terms of cytotoxicity and chemical stability as well as challenges with implementation. Polypyrrole (PPy) is a widely studied conductive polymer known for its strong biocompatibility in vitro and in vivo, good chemical stability in water, and high conductivity at physiological conditions<sup>1</sup>; however, PPy has been used in only limited applications involving cardiac cell types<sup>2</sup>. Elucidating the behavior of cardiomyocytes on PPy-containing substrates is an important step in establishing the feasibility of PPy for the creation of engineered heart tissue.

**Methods:** In this study, uniform scaffolds were prepared from sodium hydroxide-treated polycaprolactone (PCL) or interpenetrating fibers of PPy grown within PCL (PPy-PCL). Incubation times of PCL in sodium hydroxide were varied between 0 and 48 h to determine optimal conditions for growth and attachment of cardiomyocytes. HL-1 cardiomyocytes were cultured on the surface of each scaffold for up to 10 days prior to assessment of cellular attachment and viability using fluorescent imaging. Gap junction gene expression, gap junction formation, and calcium handling (calcium transient velocity and duration) of the cardiomyocytes was examined using quantitative PCR, immunocytochemistry, and optical mapping, respectively.

**Results:** PPy-PCL scaffold resistivity ( $1.0 \pm 0.4 \text{ k}\Omega \text{ cm}$ ) was similar to that of native heart tissue, whereas PCL resistivity was infinite. Both the PCL and PPy-PCL substrates proved effective at supporting attachment and viability of cardiomyocytes. Higher numbers of adherent cardiomyocytes per unit area were observed on PCL with increasing duration of exposure to NaOH ( $1,568 \pm 126 \text{ cells mm}^{-2}$ ,  $2,880 \pm 439 \text{ cells mm}^{-2}$ ,  $3,623 \pm 456 \text{ cells mm}^{-2}$  for 0, 24, 48 h of NaOH treatment, respectively; PPy PCL:  $2,434 \pm 166 \text{ cells mm}^{-2}$ ). Similar numbers of viable cardiomyocytes (~90%) were quantified on each substrate. Expression of the gap junction protein, connexin-43 (Cx43), remained unchanged between materials. Cx43 localization did differ between cells on PPy-PCL versus on PCL, with larger numbers of cardiomyocytes having Cx43 around their periphery when cultured on PPy-PCL relative to those cultured on PCL ( $60.3 \pm 4.3\%$  vs.  $46.6 \pm 5.7\%$ ). Additionally, the velocity of calcium wave propagation increased and calcium transient duration (50%) decreased for cardiomyocyte cell sheets on PPy PCL ( $1612 \pm 143 \text{ }\mu\text{m/s}$ ,  $910 \pm 63 \text{ ms}$ ) relative to cells on PCL ( $1129 \pm 247 \text{ }\mu\text{m/s}$ ,  $1129 \pm 24 \text{ ms}$ ).

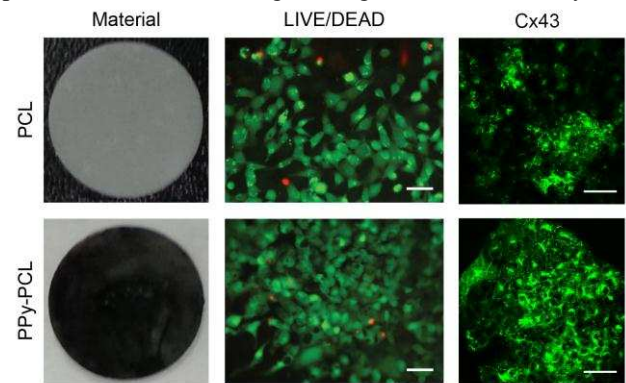


Figure 1. (Left) Representative images of PCL and PPy-PCL. (Center) Viability was consistent among HL-1 cells cultured on each scaffold. (Right) HL-1 cells on PPy-PCL demonstrated higher peripheral Cx43 localization.

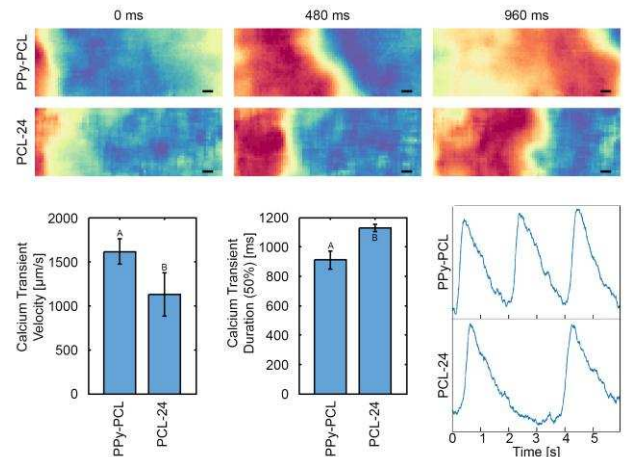


Figure 2. (Top) Representative calcium wave propagation across HL-1 monolayers on PCL and PPy-PCL. The velocity of calcium wave propagation was significantly faster (Bottom-left) and calcium transient durations were significantly lower (Bottom-center) for CM monolayers grown on PPy-PCL. (Bottom-right) Representative calcium traces from each scaffold.

**Conclusions:** This study demonstrated that conductive PPy-PCL films effectively support cardiomyocyte culture. PPy-PCL substrates promoted cellular attachment at comparable densities and cell areas relative to NaOH-treated PCL films and without a change in cellular viability. Cx43 gene expression was similar for HL-1 cardiomyocytes grown on PPy-PCL and PCL films. However, localization of Cx43 protein differed. Additionally, HL-1 cell sheets grown on conductive PPy-PCL films supported significantly faster calcium wave propagation and significantly lower calcium transient durations relative to HL-1 cell sheets grown on control PCL films. Therefore, PPy-PCL may provide effective cardiogenic tissue engineering scaffold.

## References:

<sup>1</sup>Balint R. Acta Biomater. 2014;10(6):2341-53.

<sup>2</sup>Kai D. J Biomed Mater Res A. 2011;99(3):376-85.